USER GUIDE

Mondrian™ SP Universal Cartridge

PART NO. 8010

Developed and Manufactured for NuGEN by Advanced Liquid Logic

NuGEN
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I. Introduction

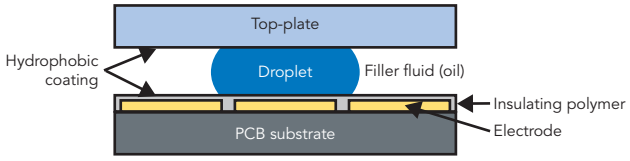
A. Background

The Mondrian™ SP Universal Cartridge kit is provided for use with protocols that use sample preparation reagents from third party vendors and includes a single use, disposable cartridge designed to facilitate hands-free digital microfluidics genomic sample preparation on the Mondrian SP Workstation. The SP Cartridge is used in all Mondrian SP protocols and offers a convenient 8-sample batch size, simple reagent loading and easy recovery of sample. Reagents are contained in discrete droplets encased within filler fluid, isolating the reactions from the lab environment and carry-over contamination.

B. Construction

Cartridges prepared for use consist of an oil layer (Filler Fluid) sandwiched between two substrates onto which insulated electrodes are patterned. By changing the relative voltages of the patterned electrodes, aqueous droplets are manipulated to perform complex assays. Droplets are dispensed from loading ports, transported to various locations on the cartridge, mixed, incubated and collected using only software control. Figure 1 shows a cutaway side view schematic of a cartridge. Figure 2 shows a top view of the cartridge.

Figure 1. Cartridge cutaway side view.



I. Introduction

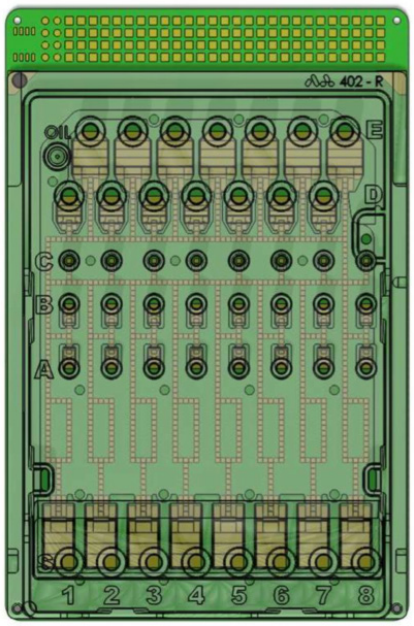
C. Cartridges

Cartridges are unpacked, inserted into the workstation, loaded with filler fluid, samples and reagents, and a run is initiated through the touch screen user interface. Cartridges are one-time-use only. Cartridge re-use is not supported even if a run is canceled prior to completion.

To ensure proper assay performance, cartridge loading procedure must be strictly followed. Consult this Mondrian SP Universal Cartridge User Guide and appropriate Application Note or Application User Guide for proper cartridge loading and setup procedure to achieve optimum results.

Figure 2 shows a diagram of a Mondrian SP Cartridge.

Figure 2. Top view of the cartridge. Sample ports along the bottom edge are labeled S1–8. Other reagent port rows are labeled A–E. Note that there are 8 ports in rows A–C, and 7 in rows D and E. There is a port labeled "OIL," sized for a standard Luer-lock connector, where the Filler Fluid is added to the cartridge. The cartridge electrodes are visible at the top of the cartridge as rows of small gold squares.



II. Kit Components

The Mondrian SP Universal Cartridge kit is shipped with ancillary consumables for use with third party reagents as detailed in the appropriate Application Note (contact NuGEN Technical Services at techserv@nugen.com) to perform complex sample preparation assays on the Mondrian SP Workstation. Protocol-specific loading guides that can be overlayed on the cartridge are also provided. Table 1 details the kit contents. All components should be stored at room temperature.

Table 1. Additional Reagents and Components Supplied with Mondrian SP Universal Cartridge.

MONDRIAN SP UNIVERSAL CARTRIDGE KIT	8010-08 PART NUMBER	8010-32 PART NUMBER
Pack Size	**1 cartridge of 8 samples/cartridge**	**4 cartridges of 8 samples/cartridge**
Reagent Additive	S01555	S01586
Sample Concentration Solution	S01556	S01587
Bead Binding Solution	S01557	S01588
Bead Wash Solution	S01558	S01589
Elution Buffer	S01559	S01590
Filler Fluid	S01561	S01561
SP Cartridge (empty)	P01190	P01190
Cartridge Loading Guide(s)	P01189	P01189

III. Operation

A. Filling the Cartridge

SP Cartridges must be filled with the provided Filler Fluid prior to loading with reagents. Cut open the foil packet containing the cartridge, remove the cartridge from the packet and place on lab bench. Alternatively, the cartridge can be inserted into the deck of the Mondrian SP Workstation and locked into place with the cartridge lever prior to filling and loading. See Section III.C. for instructions on inserting the cartridge into the Mondrian SP Workstation.

Important: Care should be taken when removing the cartridge from the packaging. Handle the cartridge by the sides and the non-electrode end. Do not handle the cartridge by the electrodes.

Remove the tube of Filler Fluid from the kit box and gently twist off the bottom cover, exposing the Luer-lock tip. Carefully insert the tip onto the port marked "OIL" on the cartridge while twisting the tube in a clockwise direction as you do this to firmly seat the cartridge on the port. Once the tube is firmly seated such that it remains upright in place, carefully snap off the top cover from the tube, taking care that the Filler Fluid tube remains upright and connected to the cartridge. Filler Fluid will begin to flow into the cartridge. The Filler Fluid will come partway up the sample and reagent ports, but will not overflow onto the cartridge as long as the cartridge remains level. Carefully remove the Filler Fluid tube from the cartridge while holding the cartridge in place on the bench top and discard the empty Filler Fluid tube. If some of the ports do not appear to have filler fluid partway up the port, gently tap or very gently shake the cartridge on the bench top, keeping the cartridge level, and avoid spilling Filler Fluid out of the cartridge.

B. Cartridge Quality Control Check

The Mondrian SP Cartridge QC protocol is a QC check that we recommend be performed prior to adding samples and reagents to the Mondrian SP Cartridge. This QC check confirms the functionality of the Mondrian SP Cartridges prior to use.

The Mondrian SP Cartridge QC protocol requires inserting the Filler Fluid-containing cartridge into the deck of the Mondrian SP Workstation. Some customers may find it easier to place the fluid-containing cartridge on the workstation and then pipet the Elution Buffer into the cartridge. Alternatively, it may be easier to pipet the Elution Buffer into the fluid-containing cartridge (while the cartridge rests on the bench top) and then insert the reagent and fluid-filled cartridge into the deck of the Workstation (moving carefully to avoid spilling the fluid or disturbing the reagent).

1. Locate the Cartridge Loading Guide that is provided with each Mondrian SP Cartridge, and place the loading guide on the cartridge (Note: it is only possible to place the loading guide on the cartridge in a single orientation).

2. **Optional:** Carefully move the Filler Fluid-containing cartridge (and its Cartridge Loading Guide) to the deck of the Mondrian SP Workstation and insert the cartridge into the deck.
3. Remove the Elution Buffer reagent tube (shipped and stored at room tempeerature) from the Mondrian SP Universal Cartridge kit:
   - Part # S01559 in the Mondrian SP Universal Cartridge 1-pack (part # 8010-08), *or*
   - Part # S01590 in the Mondrian SP Universal Cartridges 4-pack (part # 8010-32).
4. Set a 50 or 100 µL single channel pipette to 50 µL and remove 50 µL of Elution Buffer from the tube.
5. Load 50 µL of Elution Buffer into port E5 of the Mondrian SP Cartridge. When adding the reagent to the cartridge, place the tip of the pipette below the surface of the filler fluid and slowly depress the plunger. Dispense the reagent completely and carefully pull the pipette tip back out.

   Note: Do NOT add any samples or other reagents to the cartridge at this time. Ensure that only Elution Buffer has been loaded.
6. If the cartridge is not already on the Mondrian SP Workstation deck, carefully transport the cartridge to the Mondrian SP Workstation and insert the cartridge into the deck.
7. If not already ON, locate the instrument ON/OFF switch at the back of the instrument and turn ON.
8. Press the 'On' button (Figure 3) on the front of the instrument to turn the instrument on.

Figure 3. The Mondrian SP Workstation 'On' button, located on the front of the instrument

9. Pull the cartridge lever of the Mondrian SP Workstation forward to the locked position and close the lid of the workstation.
10. Select 'Run' on the touch screen menu, choose the Mondrian SP Cartridge QC protocol from the list of protocols and then select 'Next' to proceed to the Protocol Information screen.
11. Select 'Next' to proceed to the Run Information screen.
12. **Optional:** Enter run details as required in the Run Information screen.
13. Select Next to proceed to the Run Confirmation screen and select 'Start Run'.

III. Operation

The Mondrian SP Cartridge QC protocol will take about nine minutes to complete. During this test, Elution Buffer droplets will be dispensed from the E5 reservoir and transported around the cartridge prior to being discarded. The purpose of this test is to confirm the successful transport of droplets across all lanes of the cartridge.

At the end of the protocol, the instrument will display the Run Complete screen and one of the following messages:

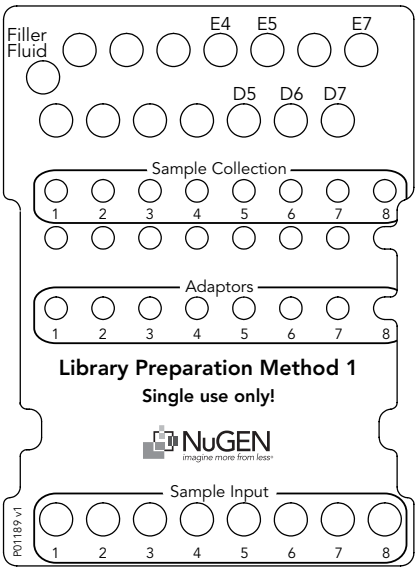
MESSAGE	MEANING	NEXT STEP
Mondrian SP cartridge passed. Continue to intended protocol.	No errors detected. Droplet transport was normal.	Press 'OK' on the Run Complete screen to return to the main menu. Proceed to section C: Loading Samples and Reagents (below) in the Mondrian SP Universal Cartridge user guide or follow the instructions (for 3rd party reagents) in the appropriate applications note. The user may remove the cartridge from the Workstation to load samples and remaining reagents on the bench top, taking care that the cartridge remains level at all times. **Note:** It is not necessary to add additional Elution Buffer or Filler Fluid to the cartridge prior to use.
Mondrian SP cartridge failed. Remove cartridge from instrument deck and set aside prior to contacting NuGEN Technical Support.	A problem was detected with droplet transport within the cartridge.	Press 'OK' on the Run Complete screen to return to the main menu. Remove the cartridge from the deck and set it aside (do not discard) and contact NuGEN Technical Support for further instructions. Users who proceed with SP protocols and/or who load additional SP reagents and samples onto failed cartridges are doing so at their own risk and will not be compensated for loss of reagents, samples or cartridges by NuGEN Technologies, Inc.
Mondrian SP cartridge status is undetermined. Please consult Mondrian SP Universal Cartridge User Guide or appropriate NuGEN® SP Library Systems User Guide for further instructions.	The results are inconclusive and must be repeated one more time prior to making a determination on the quality of the cartridge.	Repeat the Mondrian SP Cartridge QC protocol one more time. • Do not add any additional Filler Fluid or Elution Buffer to the cartridge. • Press OK on the Run Complete screen to return to the main menu. • Proceed to re-run the Mondrian SP Cartridge QC protocol starting from Step 10 in the protocol above. If the message after the second Mondrian SP Cartridge QC protocol is: • 'Mondrian SP cartridge passed,' then proceed as outlined in Next Step for passing cartridges (above). • 'Mondrian SP Cartridge failed' or 'Mondrian SP cartridge status is undetermined,' do not use the cartridge and contact NuGEN Technical Support.

C. Loading Samples and Reagents

Sample and Reagent addition can be performed either while the cartridge is resting on the bench top or in the deck of the Mondrian SP Workstation. See Section III.C. for instructions on inserting the cartridge into the Mondrian SP Workstation. Detailed instructions for loading reagents and samples are application specific. Many sample preparation protocols may appear to be similar. Verify that you are referring to the User Guide specific to the sample preparation protocol you intend to run, and that you have obtained all of the necessary additional reagents and materials prior to loading the cartridge. Contact NuGEN Technical Services at techserv@nugen.com for more information.

Ensure that samples and reagents are correctly loaded into the cartridge before starting the run. Use the application-specific loading guides to aid you in proper placement of reagents and samples. An example of the Library Preparation Method 1 loading guide is shown in Figure 4.

Figure 4. An example cartridge loading guide. Guide pictured is for Library Preparation Method 1.



When adding sample or reagent, place the tip of the pipette below the surface of the filler fluid and slowly depress the plunger. When adding reagents to the smaller ports (labeled A, B or C on the cartridge, refer to Figure 2 above), guide the pipette tip gently all the way to the bottom of the well. Slowly depress the plunger to dispense the reagent completely and carefully pull the pipette tip back out. Adding samples or reagents too quickly can cause the loading port or adjacent ports to overflow and create the potential for sample cross-contamination. Multichannel pipettes can be used and are recommended particularly for adding samples and reagents such as library adaptors to the sample ports and ports in rows A–C.

Important: It is critical that a 10 µL pipette be used for adding any volumes of ≤5 µL. Do not use 2 µL pipettes to add reagents to the cartridge as these will not generate sufficient force to expel the reagents from the pipette tip into the filler fluid-containing cartridge.

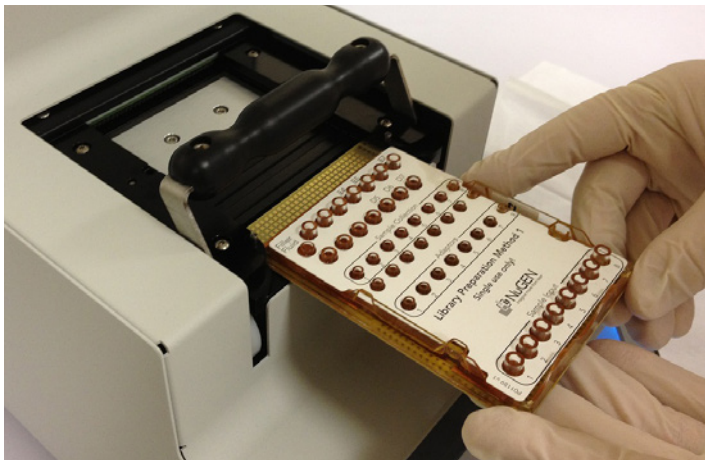
D. Cartridge Insertion

1. Raise the lid of the Mondrian SP Workstation to reveal the cartridge deck (Figure 5).

Figure 5. Top view of the cartridge deck of the Mondrian SP Workstation.

2. With the cartridge lever in the up position (lifted and moved toward the rear of the deck) hold the cartridge level with the plastic top cover with ports facing up and the gold colored electrodes pointing towards the Workstation. Carefully insert the cartridge into the cartridge deck flush with the surface of the deck, allowing the side rails to guide it into place (see Figure 6). If you encounter any resistance to the cartridge moving into the deck, carefully inspect for any foreign objects that are impeding the cartridge and remove them. If the cartridge appears to be catching on the edge of the heater bars or Peltier cooling plate, you may gently press down on them to allow the cartridge to move forward. The heater bars and cooling unit are designed to move up and down slightly in order to firmly engage the cartridge when the cartridge lever is in the down and locked position. However, do not press too hard on the heater bars or Peltier unit as this may cause them to become misaligned.

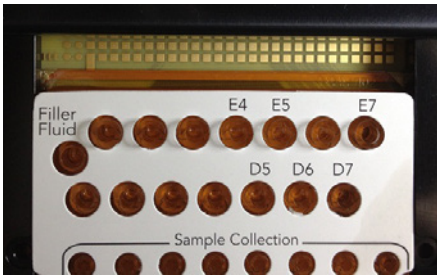
Figure 6. Inserting the cartridge.



3. Confirm that the cartridge fits snugly into the rear of the deck and that the electrodes are not visible from above (Figure 6). This will ensure that the electrodes will make good contact with the control electronics at the rear of the deck. When the cartridge is properly inserted, three guide arrows etched into the metal surface at the front edge of the deck should be visible with their tips flush with the edge of the cartridge, as illustrated in Figure 8.

Figure 7. Determining if the SP Cartridge is properly loaded in the deck. If the electrodes are still visible after the cartridge has been loaded (A), the cartridge loading has not been completed, and the cartridge should be pushed further back until no electrodes are visible from above (B).

A



B

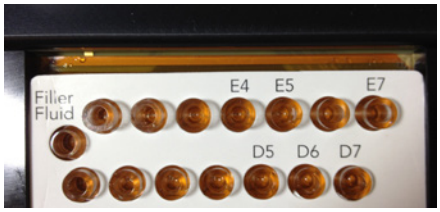
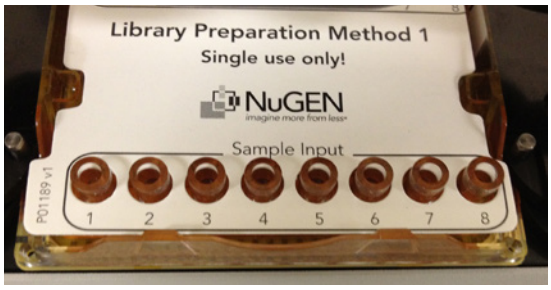


Figure 8. Gently insert the cartridge into the deck until the front edge of the cartridge is aligned with the tips of the machined arrows. Do not stop inserting the cartridge when the front edge is flush with the edge of the deck (A) but continue to insert the cartridge until the edge aligns with the tips of the arrows (B).

A



B

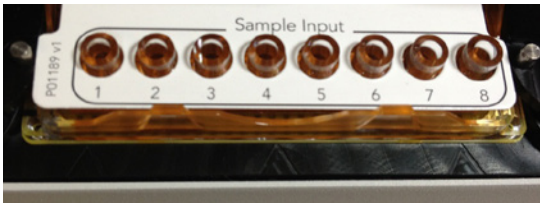
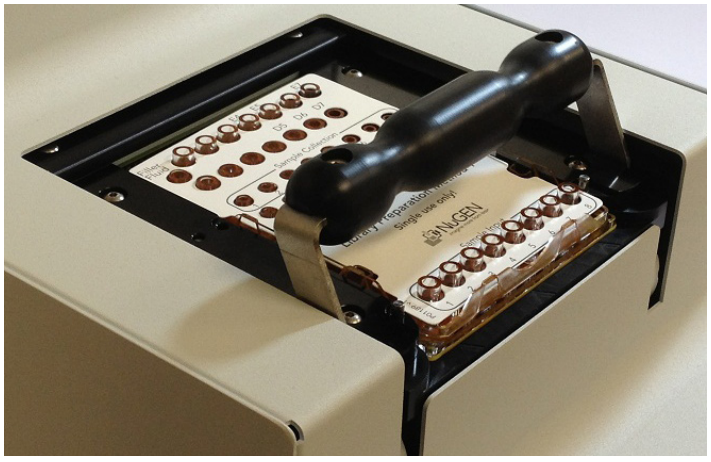


Figure 9. Cartridge inserted into deck.



4. Pull the locking lever forward and down as illustrated in Figure 10 to engage the control electronics and lock the cartridge into place.

Figure 10. Locking the cartridge deck.

Note: Once a run has started, do not raise the lever as this will result in an unrecoverable interruption of the running protocol and an error flag. The Mondrian SP Workstation will not run with the lever in the up position.

Note: Filler Fluid addition and loading of the cartridge may take place either with an empty cartridge inserted into the Mondrian SP Workstation and locked into place or on the bench top with subsequent insertion of the cartridge into the Mondrian SP Workstation. Once a fully loaded cartridge has been inserted into the Mondrian SP Workstation, close the lid and proceed with running the desired protocol.

See the Mondrian SP Workstation User Manual and the appropriate Application Note or SP Reagent System User Guide for instructions on protocol selection and starting the sample preparation run.

E. Collection of Finished Product

1. Once the protocol run has completed, raise the lid and prepare all necessary materials for sample collection from the cartridge as directed in the appropriate Application Note or SP Reagent User Guide.
2. Select a 100 or 200 µL pipette and set it to a volume of 20 µL. Depress the plunger on the pipette and insert the tip all the way to the bottom of the extraction port, perpendicular to the cartridge. Make sure that you make contact with the bottom of the cartridge.
3. Maintain contact of the pipette tip with the bottom of the cartridge and release the plunger. Lift the pipette off the bottom of the cartridge to release the vacuum that has been created, drawing filler fluid and the sample droplet into the pipette tip.
4. Examine the pipette tip to ensure that the appropriately sized droplet is suspended in the Filler Fluid in the pipette tip.

5. Dispense the collected sample into the sample vessel as directed in the documentation for your particular protocol. If you do not see the sample droplet in the pipette tip, dispense the contents of the pipette tip into the sample collection vessel and repeat the sample collection procedure for the port. Sometimes it is necessary to repeat the sample collection. Note that the droplet occasionally breaks into pieces and becomes more difficult to observe. The excess filler fluid collected during the procedure will not interfere with downstream molecular processes.
6. After all the finished products have been collected from the cartridge, push the locking lever away from you to disengage the cartridge and remove the cartridge from the instrument deck by pulling it forward (if the cartridge was not already removed prior to the collection of the finished products). Dispose of the cartridge as appropriate in laboratory waste.

IV. Technical Support

For Technical Support, please contact NuGEN at (U.S. only) 888.654.6544 (Toll-Free Phone) or 888.296.6544 (Toll-Free Fax) or email techserv@nugen.com.

In Europe contact NuGEN at +31(0)135780215 (Phone) or +31(0)135780216(Fax) or email at europe@nugen.com.

In all other locations, contact your NuGEN distributors Technical Support team.

V. Appendix

A. Troubleshooting

In the event of a performance issue with the Mondrian SP System, the most likely cause is due to incorrect loading of the cartridge or the insertion of the cartridge into the deck of the Workstation. Ensure that you follow directions carefully and ensure that all reagents and samples have been loaded into the correct locations. Failed reactions may also result from electrodes that have become fouled with Filler Fluid. See the Mondrian SP Workstation User Manual (document # M01264) for instructions on cleaning of the electrodes. For other issues please contact NuGEN Technical Support at techserv@nugen.com.

B. Update History

This document, the Mondrian SP Universal Cartridge User Guide (M01265v2.1) is an update to address the following topics:

DESCRIPTION (VERSION)	SECTION	PAGE(S)
Update patent and copyright statement, warranty statement and URLs	Throughout	Throughout

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